

WE CLAIM:

1. A non-transgenic herbicide resistant plant, which plant expresses a mutant EPSPS gene product and which plant has substantially normal growth as compared to a  
5 plant expressing the wild-type EPSPS gene product.
2. A non-transgenic herbicide resistant plant, which plant expresses a mutant EPSPS gene product, which gene product has substantially the same level of catalytic activity as compared to the wild-type gene product.
- 10 3. The plant according to claim 1 or 2 in which the herbicide is a member of the phosphonomethylglycine family.
4. The plant according to claim 3 in which the member of the  
15 phosphonomethylglycine family is glyphosate.
5. The plant according to claim 1 or 2 in which the EPSPS gene is mutated at one or more amino acid positions, said positions selected from the group consisting of Leu<sub>173</sub>, Gly<sub>177</sub>, Thr<sub>178</sub>, Ala<sub>179</sub>, Met<sub>180</sub>, Arg<sub>181</sub>, Pro<sub>182</sub>, Ser<sub>98</sub>, Ser<sub>255</sub> and Leu<sub>198</sub> in *Arabidopsis* or  
20 at an analogous amino acid residue in an EPSPS paralog.
6. The plant according to claim 5 in which the positions in the *Zea mays* paralog are selected from the group consisting of Leu<sub>97</sub>, Gly<sub>101</sub>, Thr<sub>102</sub>, Ala<sub>103</sub>, Met<sub>104</sub>, Arg<sub>105</sub>, Pro<sub>106</sub>, Ser<sub>223</sub>, Ser<sub>179</sub> and Leu<sub>122</sub>.
- 25 7. The plant according to claim 5 in which the positions in the *Brassica napus* paralog are selected from the group consisting of Leu<sub>169</sub>, Gly<sub>173</sub>, Thr<sub>174</sub>, Ala<sub>175</sub>, Met<sub>176</sub>, Arg<sub>177</sub>, Pro<sub>178</sub>, Ser<sub>94</sub>, Ser<sub>251</sub> and Leu<sub>194</sub>.
- 30 8. The plant according to claim 5 in which the positions in the *Petunia hybrida* are selected from the group consisting of Leu<sub>169</sub>, Gly<sub>173</sub>, Thr<sub>174</sub>, Ala<sub>175</sub>, Met<sub>176</sub>, Arg<sub>177</sub>, Pro<sub>178</sub>, Ser<sub>94</sub>, Ser<sub>251</sub> and Leu<sub>194</sub>.



- (iii) Thr<sub>174</sub> - Ile or Val or Leu
- (iv) Ala<sub>175</sub> - Gly
- (v) Met<sub>176</sub> - Cys
- (vi) Arg<sub>177</sub> - Leu or Ser
- 5 (vii) Pro<sub>178</sub> - Leu or Ser
- (viii) Ser<sub>94</sub> -Asp
- (ix) Ser<sub>251</sub> -Ala
- (x) Leu<sub>194</sub> -Lys.

10 13. The plant according to claim 8 in which the mutated gene results in one or more of the following amino acid substitutions in the EPSPS enzyme in comparison with the wild-type sequence:

- (i) Leu<sub>169</sub> - Phe
- (ii) Gly<sub>173</sub> - Ala or Ile
- 15 (iii) Thr<sub>174</sub> - Ile or Val or Leu
- (iv) Ala<sub>175</sub> - Gly
- (v) Met<sub>176</sub> - Cys
- (vi) Arg<sub>177</sub> - Leu or Ser
- (vii) Pro<sub>178</sub> - Leu or Ser
- 20 (viii) Ser<sub>94</sub> -Asp
- (ix) Ser<sub>251</sub> -Ala
- (x) Leu<sub>194</sub> -Lys.

25 14. A method for producing a non-transgenic herbicide resistant or tolerant plant comprising

- a. introducing into a plant cell a recombinagenic oligonucleobase to produce a mutant EPSPS gene; and
- b. identifying a cell having a mutated EPSPS gene, which cell has substantially normal growth as compared to a corresponding wild-type plant cell.

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15. A method for producing a non-transgenic herbicide resistant or tolerant plant comprising

- a. introducing into a plant cell a recombinagenic oligonucleobase to produce a

b. identifying a cell having a mutated EPSPS gene, which encoded mutant EPSPS protein has substantially the same catalytic activity as compared to a corresponding wild-type EPSPS protein.

5                   16. The method according to claim 14 or 15 in which the recombinagenic oligonucleobase is a mixed duplex nucleotide or a SSMOV.

10                   17. The method according to claim 16 in which the mixed duplex nucleotide contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of the first fragment of the target EPSPS gene and a second homologous region which has a sequence identical to the sequence of at least 6 based pairs of a second fragment of the target EPSPS gene, and an intervening region which contains at least one nucleobase heterologous to the target EPSPS gene, which intervening region connects the first and second homologous region.

15                   18. The method according to claim 14 or 15 in which the recombinagenic oligonucleobase is introduced by electroporation.

20                   19. The method according to claim 14 or 15 which the mutant EPSPS gene is mutated at one or more amino acid positions, said positions selected from the group consisting of Leu<sub>173</sub>, Gly<sub>177</sub>, Thr<sub>178</sub>, Ala<sub>179</sub>, Met<sub>180</sub>, Arg<sub>181</sub>, Pro<sub>182</sub>, Ser<sub>98</sub>, Ser<sub>255</sub> and Leu<sub>198</sub> in *Arabidopsis* or at an analogous amino acid residue in an EPSPS paralog.

25                   20. The plant according to claim 19 in which the positions in the *Zea mays* paralog are selected from the group consisting of Leu<sub>97</sub>, Gly<sub>101</sub>, Thr<sub>102</sub>, Ala<sub>103</sub>, Met<sub>104</sub>, Arg<sub>105</sub>, Pro<sub>106</sub>, Ser<sub>23</sub>, Ser<sub>179</sub> and Leu<sub>122</sub>.

30                   21. The plant according to claim 19 in which the positions in the *Brassica napus* paralog are selected from the group consisting of Leu<sub>109</sub>, Gly<sub>111</sub>, Thr<sub>112</sub>, Ala<sub>113</sub>, Met<sub>114</sub>, Arg<sub>115</sub>, Pro<sub>118</sub>, Ser<sub>94</sub>, Ser<sub>251</sub> and Leu<sub>194</sub>.

22. The plant according to claim 19 in which the positions in the *Petunia hybrida* are selected from the group consisting of Leu<sub>109</sub>, Gly<sub>111</sub>, Thr<sub>112</sub>, Ala<sub>113</sub>, Met<sub>114</sub>,

23. The plant according to claim 14 or 15 in which the plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape, turf grasses and *Brassica* sp.

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24. An isolated mutant EPSPS protein comprising the amino acid sequence depicted in SEQ ID NO:2, in which amino acid position Leu<sub>1-3</sub> is replaced with Phe, Gly<sub>1--</sub> is replaced with Ala or Ile, Thr<sub>1-8</sub> is replaced with Ile or Val or Leu, Ala<sub>1-9</sub> is replaced with Gly, Met<sub>180</sub> is replaced with Cys, Arg<sub>181</sub> is replaced with Leu or Ser, Pro<sub>182</sub> is replaced with  
10 Leu or Ser, Ser<sub>98</sub> is replaced with Asp, Ser<sub>255</sub> is replaced with Ala or Leu, Leu<sub>198</sub> is replaced with Lys, which mutant EPSPS protein has increased resistance or tolerance to a herbicide, which herbicide is a member of the phosphonomethylglycine family.

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ADD B3)

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